The Ochsner Journal 13:26–32, 2013

Prorenin Receptor in Distal Nephron Segments of 2-Kidney, 1-Clip Goldblatt Hypertensive Rats

Minolfa C. Prieto, MD, PhD, Fady T. Botros, PhD, Kimberly Kavanagh, MD, L. Gabriel Navar, PhD

Department of Physiology and Tulane Renal Hypertension Center, Tulane University School of Medicine, New Orleans, LA

ABSTRACT

Background: The prorenin receptor (PRR) is expressed in the kidneys and has been localized to mesangial cells, renal arterioles, and distal nephron segments. By binding renin or prorenin, this receptor increases renin catalytic activity and activates prorenin. The renin gene is expressed by the principal cells of collecting ducts and is enhanced in angiotensin II (AngII)-dependent hypertension and in both kidneys of 2-kidney, 1-clip (2K1C) Goldblatt hypertensive rats. Colocalization of PRR with prorenin and renin in distal nephron segments may contribute to increased local AngII formation.

Methods: We examined the specific cell-type localization of PRR in distal nephron segments and the changes in PRR gene expression in both kidneys of 2K1C hypertensive rats (n=6) and sham-operated rats (n=5).

Results: After 25 days, systolic blood pressure and plasma renin activity increased to 186 ± 8 mmHg and 12.8 ± 3 ng/AngI/mL/hr, respectively, in 2K1C rats compared to controls (133 ± 9 mmHg and 7.1 ± 1 ng/AngI/mL/hr, respectively). Immunohistochemistry of the PRR on fixed kidney sections showed intense positive staining in the apical aspects of intercalated cells in collecting ducts. PRR immunoreactivity (clipped kidney: 2.3 ± 1 IDU; nonclipped kidney: 1.3 ± 0 IDU; sham: 1.0 ± 0.0 IDU; $P<0.05$) and messenger RNA levels measured by quantitative real-time polymerase chain reaction (clipped kidney: 1.3 ± 0.1 au; nonclipped kidney: 0.9 ± 0.3 au; sham: 1 ± 0.0 au; $P<0.05$] were increased in collecting duct cells of clipped kidneys of 2K1C rats compared to nonclipped and sham kidneys.

Conclusion: The enhanced renin gene expression in the collecting ducts of hypertensive rats suggests that the renin secreted by principal cells is then anchored by the PRR on the intercalated cells, thus contributing to increased angiotensin peptide generation in distal nephron segments.

Address correspondence to
Minolfa C. Prieto, MD, PhD
Department of Physiology
Tulane University Health Sciences Center
1430 Tulane Ave., SL39
New Orleans, LA 70112
Tel: (504) 988-2445
Fax: (504) 988-2675
Email: mprieto@tulane.edu

Keywords: Gene expression, immunohistochemistry, nephrons, real-time polymerase chain reaction, renin-angiotensin system

Funding: This project was supported by the National Heart, Lung, and Blood Institute (HL-26731), the Institutional Developmental Award Program of the National Center for Research Resources (P20RR-017659), the Eunice Kennedy Shriver National Institute of Child Health & Human Development (K12HD043451), and the American Heart Association (09BGIA2280440).

Disclosures: Fady T. Botros, PhD, is currently a clinical research scientist at the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN.

INTRODUCTION

Intrarenal angiotensin II (AngII) is formed by tissue renin acting on angiotensinogen (AGT) delivered to the kidney as well by AGT produced intrarenally by the proximal tubule cells. In addition to their localization in juxtaglomerular cells, renin messenger RNA (mRNA) and protein are present in connecting tubules and in cortical and medullary collecting ducts.[1-3] Renin in the distal nephron segments is specifically expressed by principal cells[2,3] and seems to be differentially regulated from that produced by juxtaglomerular cells.[4] In response to chronic AngII infusions, renin mRNA and protein levels increase in principal cells from connecting tubules and collecting ducts, indicating stimulation of tubular renin during AngII-dependent hypertension.[3] This stimulatory effect of collecting duct renin in AngII-dependent hypertension is mediated by an AngII type 1 receptor (AT_1R) mechanism because treatment with AT_1R blockers (ARBs) prevents the stimulation of distal nephron renin.[4] Furthermore, the upregulation of renin expression in both kidneys of 2-kidney, 1-clip (2K1C) Goldblatt hypertensive rats indicates that renin stimulation occurs independently of blood pressure.[5] These recent studies support the hypothesis that inappropriate activation of renin in distal nephron segments—by acting on AGT originating from the

proximal tubular segments—leads to increases in distal intrarenal AngII formation and contributes to sodium retention and the development and progression of hypertension.[3,6]

Nguyen et al[7] cloned the prorenin receptor (PRR), a 350–amino acid protein with a single transmembrane domain that binds renin or prorenin, increasing the catalytic activity of renin and fully activating prorenin. The PRR activation also elicits intracellular signals via the extracellular signal-regulated kinase 1 and the extracellular signal-regulated kinase 2 mitogen-activated protein kinase. The PRR is expressed in the heart, brain, placenta, kidney, and liver. In the human kidney, the PRR has been localized to glomerular mesangial cells and the subendothelium of renal arteries.[7] Immunohistochemistry studies have also identified the PRR in tubular segments in normal human and rat kidneys at the basolateral side of distal tubular cells, as well as in macula densa cells.[8,9]

The coordinated presence of renin and the PRR in the distal nephron segments may provide a pathway for distal nephron angiotensin I (AngI) generation and, with further conversion by angiotensin-converting enzyme (ACE) activity present in the collecting ducts,[10,11] lead to subsequent generation of AngII in distal nephron segments. However, the exact cellular localization of the PRR in distal nephron segments and the alterations occurring in hypertension models remain unclear. In the present study, we examined the cell type localization, regional distribution, and gene expression of the PRR in normal rat kidneys and in both clipped and nonclipped kidneys from hypertensive 2K1C Goldblatt rats.

METHODS

Experimental Animals and Tissue Preparation

All experimental protocols were approved by the Tulane Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (150 to 175 g; Charles River Laboratories, Wilmington, MA) were cage housed and maintained in a temperature-controlled room with a 12-hour light/dark cycle, with free access to tap water and standard rat chow (Ralston Purina, St. Louis, MO) during the duration of the study. Following a training period for blood pressure measurements, the systolic blood pressures (SBPs) were monitored by tail-cuff plethysmography (Visitech, BP-2000, Apex, NC) 1 day before placement of a u-shaped silver clip (lumen diameter of 0.25 mm) on the left renal artery and at the end of the first, second, and third weeks of the study. This size clip has been shown to allow preservation of renal function in the clipped kidney. The surgery (n=6) was performed during anesthesia with Isoflurane (IsoSol, Abbot Laboratories, Chicago, IL). Sham operation (n=5) was performed on the control group.

Sample Collections, Tissue Preparations, and Assessments

On day 25 after unilateral renal clip surgery, trunk blood samples and both kidneys were harvested following conscious rat decapitation. One milliliter of trunk blood was collected into a chilled tube containing 5.0 mmol/L ethylenediaminetetraacetic acid and centrifuged at 4,000 rpm for 30 minutes at 4°C. The plasma fraction was then removed for plasma renin activity (PRA) assay.[12] PRA was expressed as ng/mL/hr of generated AngI. For renal tissue studies, the left (clipped kidney [CK]) and right (nonclipped kidney [NCK]) kidneys were sectioned under sterile and RNA-free conditions, and poles were immediately fixed in 4% paraformaldehyde for immunohistochemical studies. From the middle section, medullas were dissected from cortexes under a stereomicroscope and stored at –80°C in RNA*later* RNA Stabilization Reagent (Ambion, Austin, TX) until they were processed for total RNA extractions.

For real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) of the rat PRR, 20 ng of total RNA per well was extracted from the rat kidney cortex and medulla samples and quantitated as previously described[3] using the following sequences: primers (sense, 5′-ATCCTTGAGACGAAACAAGA-3′; antisense, 5′-AGCCAGTCATAATCCACAGT-3′) and probe (5′-6-FAM-ACACCCAAAGTCCCTACAACCTTG-BHQ1-3′). Data of quantitative qRT-PCR were normalized by β-actin mRNA using the following sequences: primers (sense, 5′-ATCATGAAGTGTGACGTTGA-3′; antisense, 5′-GATCTTCATGGTGCTAGGAGC-3′) and probe (5′-6-HEX-TCTATGCCAACACAGTGCTGTCT-GGT-BHQ2-3′) and expressed accordingly.

For immunohistochemistry, 3 μm paraffin-embedded kidney sections were immunostained by the peroxidase technique using an automatic robot system (Biocare Medical LLC, Concord, CA) that allowed identical incubation time for all of the microscopic slides in reagents and antibodies. Mounted kidney sections were sequentially incubated with normal Rodent Block R reagent (Biocare Medical, LLC) and primary goat polyclonal PRR antibody (ATP61P2; Abcam, Inc., Cambridge, MA) at 1:200 dilution and detected with a Goat HRP-Polymer Kit (Biocare Medical, LLC). Peroxidase activity was visualized by 3,3′-diaminobenzidine tetrahydrochloride (DAB; Biocare Medical, LLC). To assist in cell-type immunocolocalization, consecutive rat kidney sections were stained with the PRR antibody, followed by anti-aquaporin 2 antibody (AQP2 at 1:500 dilution; Calbiochem, EMD Millicore, Billerica, MA) immuno-

staining that was used as a marker of principal cells. For anti-AQP2 immunohistochemistry, an alkaline phosphatase technique using the AP Polymer Kit (Biocare Medical, LLC) and visualization with Vulcan Fast Red (Biocare Medical, LLC) as chromogen were performed. Additional studies were performed by staining the same rat kidney sections sequentially with the PRR antibody, fluorescent secondary antibody Alexa Fluor 488 Donkey Anti-Goat in green (Life Technologies, Grand Island, NY), the AQP2 antibody, and fluorescent secondary antibody Alexa Fluor 594 Chicken Anti-Rabbit in red. For imaging capture and analysis, a Nikon (Tokyo, Japan) digital camera (DS-U2/L2 USB) attached to a Nikon Eclipse 50i microscope was used, and intensities of the PRR immunoreactivities (ratio of the sum of density of positive cells per area) were semiquantified in the collecting duct cells of kidneys of sham rats and in both kidneys of 2K1C rats using the NIS Elements AR version 3.0 software, as previously described.[3] Ten different microscopic fields per tissue section per animal were analyzed. The results are expressed in intensity densitometric units (IDUs) of the relative intensity normalized to the PRR immunostaining average of the sham group.

Statistical Analysis

Results are expressed as mean ± standard error of the mean. Data were evaluated by Grubb test, followed when appropriate by paired and unpaired Student *t* test or by 1-way analysis of variance with the Fisher least significant difference test. The significance of differences among groups was defined as $P<0.05$.

RESULTS

Body Weight (BW), SBP, and PRA

The table shows the profile of BW, SBP, and PRA of the rats in this study 25 days after the renal artery clipping surgery. The BW in 2K1C rats was slightly lower than that of the sham rats 7 days after surgery (251 ± 8 g vs 236 ± 5 g, respectively). Also, 2K1C rats did not gain as much weight as sham rats during the following 2 weeks (259 ± 6 g vs 280 ± 6 g, respectively) and 3 weeks (298 ± 9 g vs 322 ± 9 g, respectively), $P<0.05$. SBP values were similar in both groups of rats at the beginning of the study (2K1C: 127 ± 4 mmHg vs sham: 119 ± 4 mmHg). However, SBP increased in 2K1C rats relative to sham-operated rats (166 ± 6 mmHg vs 135 ± 19 mmHg, respectively) by 1 week after Goldblatt surgery and continued to increase during the second and third weeks (day 14: 180 ± 8 mmHg vs 134 ± 18 mmHg, respectively; day 25: 186 ± 8 mmHg vs 133 ± 9 mmHg), $P<0.001$. At 25 days, PRA was elevated in 2K1C rats compared to sham-operated rats (12.8 ± 3 ng/AngI/mL/hr vs 7.1 ± 1 ng/AngI/mL/hr, respectively), $P<0.05$.

Table. Body Weight, Systolic Blood Pressure, and Plasma Renin Activity 25 Days After Unilateral Renal Artery Clipping

Group	n	Body Weight (g)	Systolic Blood Pressure (mmHg)	Plasma Renin Activity (ng/AngI/mL/h)
Sham	5	322 ± 9	133 ± 9	7.1 ± 1
2-Kidney, 1-Clip	6	298 ± 9*	186 ± 8**	12.8 ± 3*

AngI, angiotensin I.
*Significant ($P<0.05$) difference versus sham rats.
**Significant ($P<0.001$) difference versus sham rats.
Data are mean ± standard error of the mean.

PRR Immunohistochemistry in the Distal Nephron Segments

As shown in Figure 1, co-immunofluorescence staining at high-power magnification (oil immersion, 100×) demonstrates the positive PRR immunoreactivity in a collecting duct (Figures 1A-1C). Positive PRR immunofluorescence is mainly observed in the apical aspects of some cells of the collecting duct (color green, Figure 1A). Co-immunofluorescence with AQP2 (red, Figure 1B) identifies that cells stained with AQP2 (red, Figure 1B) are a different cell type than cells stained positive for the PRR (green, Figure 1A). The absence of colocalization after merging the 2 immunofluorescence images (Figure 1C) and the lack of co-staining of the same cell type by using the PRR (brown, Figure 1D) and AQP2 (red, Figure 1E) on consecutive sections indicate that the PRR is expressed by the intercalated cells and not the principal cells (AQP2-positive cells). Figure 1F provides a lower magnification (20×) view of the PRR immunoreactivity in cortical and medullary collecting ducts of sham kidneys, CKs, and NCKs and shows a similar pattern of expression: positive cells labeled at the apical side of the cell alternate with negative cells.

Densitometric analysis of positive PRR immunoreactivities in cortical and medullary collecting duct cells from kidney sections of sham rats and the CKs and NCKs of 2K1C rats is shown in Figure 2. PRR immunoreactivity was determined in 10 different microscopic fields/tissue section/animal using a Nikon Eclipse 50*i* microscope, 40× objective, and an integrated Nikon Digital Sight DS-U2 Camera System for image processing. The intensity of PRR immunoreactivity in both kidneys of Goldblatt rats and in sham-rat kidney sections was analyzed using NIS-Elements AR Software (version 3.0 for Windows; Nikon Instruments, Inc., Melville, NY), allowing a

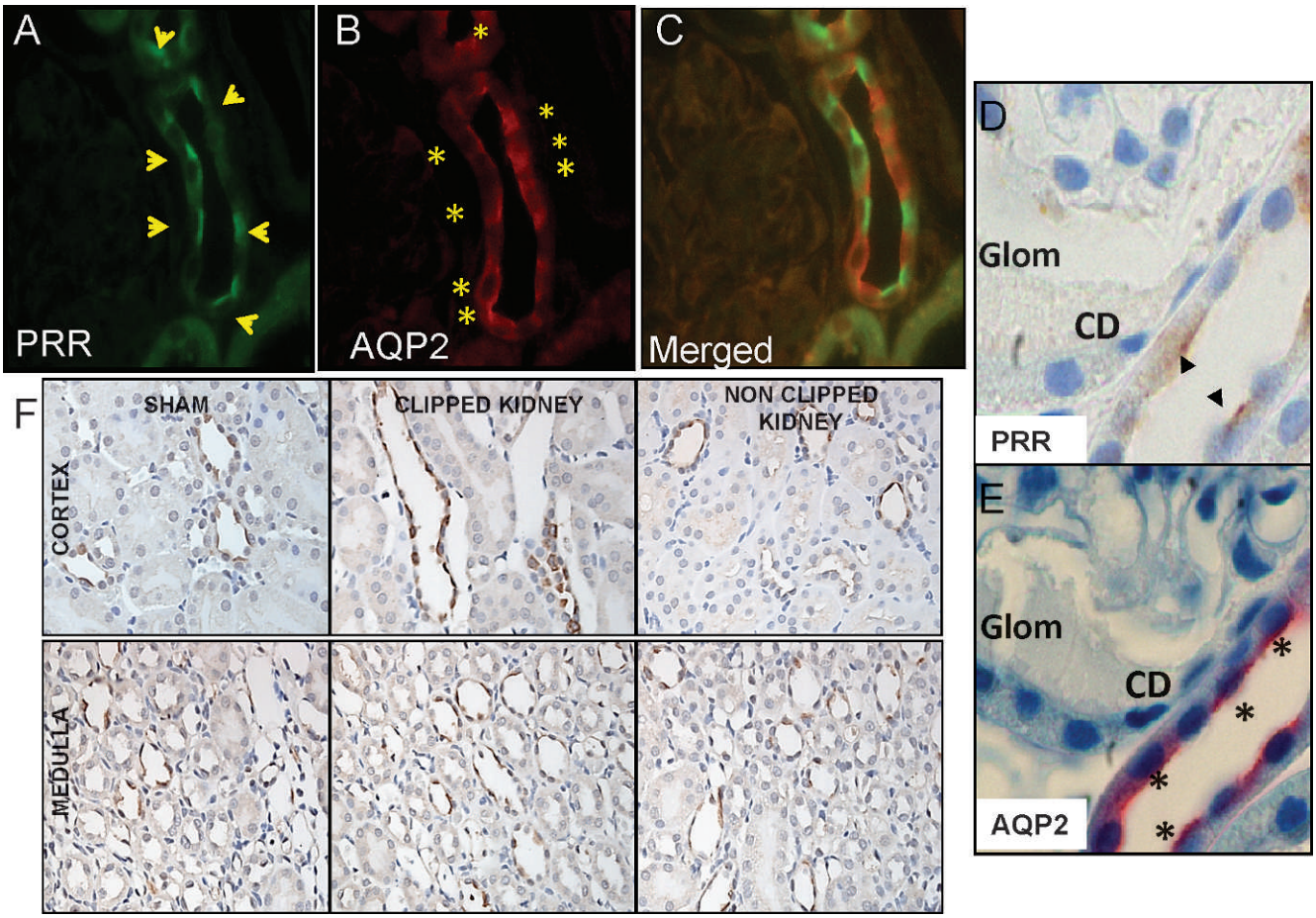

Figure 1. Prorenin receptor (PRR) immunoreactivity in rat kidneys. A: Specific PRR immunofluorenscence (Alexa Fluor 488, green) is shown on the apical aspect of some cells of a distal tubular segment. The absence of colocalization of PRR and anti-aquaporin 2 (AQP2) by using (A) immunofluorescence (PRR, Alexa Fluor 488, green) and (B) AQP2 (Alexa Fluor 594, red) on the same kidney section indicates that PRR and AQP2 are located in different cell types. Consecutive kidney sections stained by immunoperoxidase technique show (D) PRR positive staining (DAB chromogen, brown) in those cells that are (E) AQP2 negative (Vulcan Fast Red), indicating that PRR is expressed by intercalated cells in the distal nephron segment of rat kidneys. (F) Specific PRR immunoreactivity (DAB, brown chromogen) in the renal cortex (upper panels) and medulla (lower panels) from sham rats and clipped kidney and nonclipped kidney of 2-kidney, 1-clip rats is shown. Glom, glomerulus.

computerized determination of the area of positive staining (μm) and the intensity of immunoreactivity (sum of density of positive tubules in an analyzed area). For this analysis, the immunoreactivity of PRR was analyzed separately from the cortical and medullary collecting ducts. The results are expressed in IDUs of the relative intensity normalized to the PRR immunostaining average of the sham kidney. CKs of 2K1C rats exhibited higher PRR immunoreactivity in cortical and medullary collecting duct cells than sham rats and NCK rats (CK: 2.3 ± 1.0 IDU; $P<0.05$ vs sham: 1.0 ± 0.0 IDU and NCK: 1.3 ± 0.0 IDU). Immunoreactivity was also detected in mesangial cells, but its intensity was not analyzed in this study. In addition, control experiments were performed using consecutive sections incubated with normal blocking goat serum to confirm the specificity of the PRR antibody.

mRNA Levels of PRR in Renal Medullary Tissues

To obtain an index of the PRR expression in the distal nephron segments, the quantitation of its mRNA levels was performed only in renal medullary tissues to avoid contribution of PRR from glomeruli, mesangial cells, podocytes, and macula densa cells from the renal cortex. Specific rat PRR transcript levels were slightly but significantly higher in the medullary tissues of CK rats (1.3 ± 0.1 arbitrary unit [au]; $P<0.05$) but not of NCK rats (0.9 ± 0.1 au) compared to sham rats (1.0 ± 0.0 au) (Figure 3).

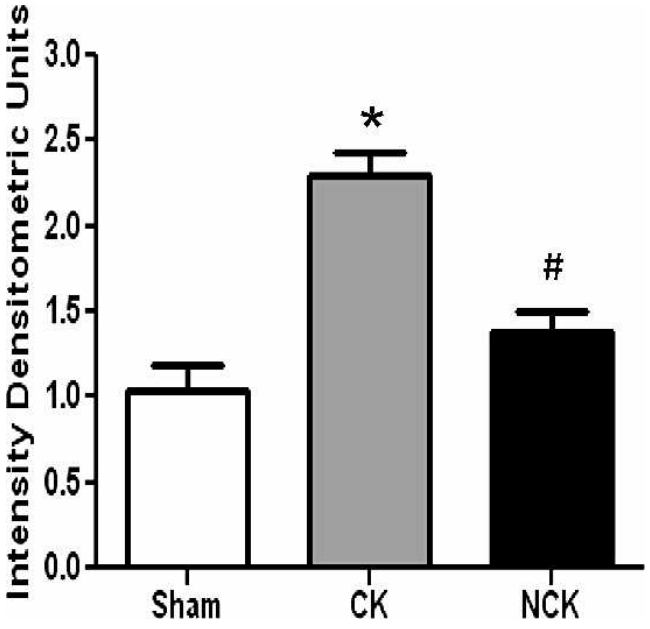

Figure 2. Semiquantitation of prorenin receptor (PRR) immunoreactivity in collecting duct cells. Densitometric analyses of the PRR intensity immunoreactivity in collecting duct cells of sham rats and clipped (CKs) and nonclipped kidneys (NCKs) of Goldblatt rats performed in 4 kidney sections/animal (10 microscopic fields/kidney sections at the renal cortex and medulla regions). Sham rats, n=5; 2-kidney, 1-clip rats, n=6. Values are mean ± standard error of the mean. Values are expressed in intensity densitometric units (IDUs); *$P<0.05$ versus sham rats. PRR antibody concentration 1:200. #$P<0.05$ CK versus NCK.

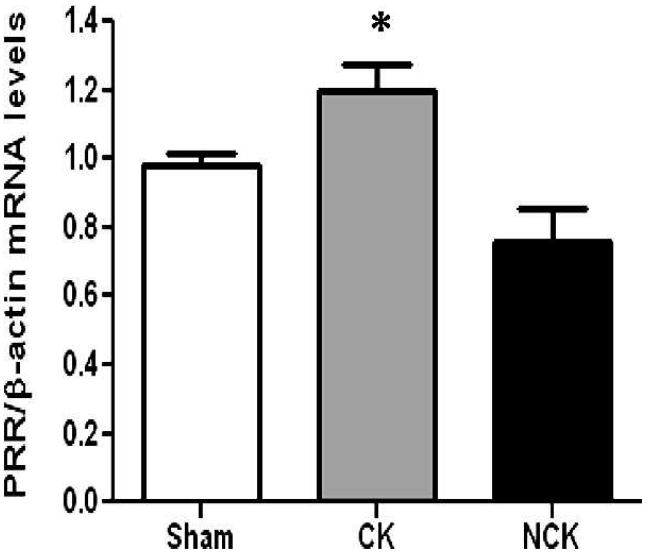

Figure 3. Kidney medullary prorenin receptor (PRR) messenger RNA (mRNA), measured by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). PRR qRT-PCR showed higher mRNA levels in the medullas of clipped kidneys (CKs) compared to nonclipped kidneys (NCKs) and sham kidneys. The mRNA levels for the target gene were determined in each sample in triplicate and expressed relative to β-actin in arbitrary units (au). *$P<0.05$ versus sham rats.

DISCUSSION

This study demonstrates that the PRR is immunoexpressed in cortical and medullary collecting ducts of normal rat kidneys and in both CKs and NCKs of hypertensive 2K1C Goldblatt rats. The cell-type immunolocalization of the PRR appears to be restricted to the intercalated cells of this renal segment. In addition, this study shows a PRR gene expression upregulation in the CK of the Goldblatt hypertensive rats, indicating a parallel upregulation of renin and PRR in distal nephron segments of the CK.

Receptor-bound renin exhibits 5-fold increased catalytic activity, and receptor-bound prorenin exhibits full enzymatic activity comparable to that of active renin.[7] Initially, Nguyen and associates[7] reported the presence of this receptor predominantly in glomerular mesangial cells and in vascular smooth muscle cells of renal arteries and coronary arteries visualized by immunofluorescence on frozen tissues. However, immunohistochemistry and in situ hybridization studies have reported that the main site of synthesis of PRR in the kidneys of normal rats and humans is at the distal tubules. [8,9]

The present study, using immunofluorescence and immunoperoxidase techniques, confirms the predominant immunoexpression of the PRR in AQP2-negative cells specifically expressed on the apical aspects of the intercalated cells of cortical and medullary collecting ducts. Advani et al[8] and Gonzalez et al[9] previously reported this renal localization. The presence of the PRR on the cell surface of the intercalated cells and not on the principal cells supports previous suggestions that renin in the principal cells of the collecting duct is locally synthesized and not caused by uptake via its receptor.[3,6,9] The immunolocalization of the PRR at the apical side of the cells in the distal nephron segments might be of great relevance in light of new findings of renin synthesis and secretion from the principal cells of the collecting ducts.[2,3,6]

We propose that the PRR located on the surface of the intercalated cells may play a pivotal role in increasing the efficiency of intratubular angiotensin peptide generation by the distal nephron segments via anchoring of the renin or prorenin synthesized and released by principal cells. Nguyen et al[7] also described the immunoexpression of this receptor on the basolateral side of distal tubular cells as well as on the macula densa cells, which may be particularly significant in regulating interstitial AngII levels. In-

creases in renal interstitial fluid AngII levels have been reported for 2 models of hypertension. Siragy and Carey[13] found that renal interstitial AngII is increased in the wrapped kidney of rats with 2-kidney, 1-wrap Grollman hypertension. Nishiyama et al[14,15] reported that renal interstitial fluid AngII concentrations are increased in rats infused with AngII for 2 weeks. Because the renal interstitial values are greater than what can be explained on the basis of equilibration with the plasma concentrations, it has been suggested that local regulation of AngII formation in the renal interstitial compartment and enhanced production of interstitial AngII might be secondary to specialized AngII-forming pathways or accumulation mechanisms.[15] The presence of the PRR at the basolateral side of distal tubular segments cells may contribute to the pool of AngII in this renal compartment.

To investigate further the mechanisms underlying the maintained capability to form intrarenal AngII in both kidneys of the 2K1C Goldblatt rat model regardless of the suppression of renin produced by the juxtaglomerular cells, we also analyzed the gene expression of the PRR in both kidneys of 2K1C rats. During 2K1C Goldblatt hypertension, a cascade of events initiated by the increases of renin secretion from the CK is followed by early increases in circulating AngII that ultimately inhibit juxtaglomerular renin synthesis and release in the NCK. High-circulating AngII levels return to normal after 2-3 weeks of unilateral renal clipping.[16] We have suggested that increased renin immunoreactivity and protein levels in the collecting duct cells with augmented mRNA renin levels in the renal medullary tissues of both CKs and NCKs from the 2K1C rats indicate enhanced local synthesis and stimulation of renin in the distal nephron segments independent of blood pressure. Importantly, in this model of hypertension we also found that renin in the medullary tissues of both kidneys of 2K1C hypertensive rats is mostly active.[5]

In the present study, we show that PRR gene expression is augmented in the CK but not in the NCK of 2K1C hypertensive rats. Krebs et al[17] also reported an upregulation of PRR in the CKs of Goldblatt rats associated with a redistribution and upregulation of renin upstream from the glomerulus in preglomerular vessels and in distal tubules.[17] The researchers did not study the NCKs. More recently, however, Muller et al[18] did not find differences in PRR gene expression in either the CKs or the NCKs compared with sham controls.

We have also demonstrated that the PRR is augmented in the collecting ducts of rats infused with AngII during 14 days[9] and in Cyp1a1Ren2-transgenic rats.[19] In addition, a soluble form of the PRR [s(P)RR] has been described[20] and found augmented in the urine of chronic AngII hypertensive rats.[9] Urine collected from AngII-infused rats incubated with recombinant prorenin showed higher AngI formation correlated with the augmentation of s(P)RR and the augmentation of the PRR mRNA levels.[9] Augmented renin synthesis and secretion by the principal cells of the collecting ducts can combine with the membrane-bound PRR and/or bind to the s(P)RR in the tubular fluid, leading to AngI formation. Thus, regardless of whether the PRR is upregulated or not in the nonclipped kidney of the 2K1C hypertensive rat model, its presence in the distal nephron segments cannot be neglected. We suggest that in the 2K1C Goldblatt rat hypertension model—particularly in the NCK—and in the chronic AngII rat[9] and the Cyp1a1-Ren2 transgenic rat[19] models, the presence of PRR in this segment of the nephron may contribute substantially to the continued capability to form AngI by binding renin or prorenin locally synthesized and augmented in the collecting duct cells of 2K1C hypertensive rats.[5,6,17]

CONCLUSIONS

Recent investigations of the intrarenal renin-angiotensin system have raised our awareness about its multiple components that are regulated independently from the systemic renin-angiotensin system. We propose that in AngII-dependent hypertension, augmented proximal tubular secretion of angiotensinogen into the lumen spills over to the distal nephron and that the coordinated actions of enhancement of renin and the PRR in distal nephron segments further enhance intratubular AngII formation in distal nephron segments, thus allowing for greater distal tubular sodium reabsorption.[6] Reduced sodium excretion contributes to the development and progression of hypertension. The luminal localization of the PRR suggests the intriguing possibility of serving to anchor renin and/or prorenin, thus preventing or minimizing its washout into the urine.

ACKNOWLEDGMENTS

We thank Dale Seth, MS, for his excellent technical assistance and Debbie Olavarrieta for preparation of the manuscript.

REFERENCES

1. Hackenthal E, Paul M, Ganten D, Taugner R. Morphology, physiology, and molecular biology of renin secretion. *Physiol Rev*. 1990 Oct;70(4):1067-1116.
2. Rohrwasser A, Morgan T, Dillon HF, et al. Elements of a paracrine tubular renin-angiotensin system along the entire nephron. *Hypertension*. 1999 Dec;34(6):1265-1274.

3. Prieto-Carrasquero MC, Harrison-Bernard LM, Kobori H, et al. Enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. *Hypertension*. 2004 Aug;44(2):223-229. Epub 2004 Jun 28.
4. Prieto-Carrasquero MC, Kobori H, Ozawa Y, et al. AT1 receptor-mediated enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. *Am J Physiol Renal Physiol*. 2005 Sep;289(3):F632-F637. Epub 2005 May 3.
5. Prieto-Carrasquero MC, Botros FT, Pagan J, et al. Collecting duct renin is upregulated in both kidneys of 2-kidney, 1-clip Goldblatt hypertensive rats. *Hypertension*. 2008 Jun;51(6):1590-1596. Epub 2008 Apr 21.
6. Navar LG, Kobori H, Prieto MC, Gonzalez-Villalobos RA. Intratubular renin-angiotensin system in hypertension. *Hypertension*. 2011 Mar;57(3):355-362. Epub 2011 Jan 31.
7. Nguyen G, Delarue F, Burcklé C, et al. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest*. 2002 Jun;109(11):1417-1427.
8. Advani A, Kelly DJ, Cox AJ, et al. The (Pro)renin receptor: site-specific and functional linkage to the vacuolar H+-ATPase in the kidney. *Hypertension*. 2009 Aug;54(2):261-269. Epub 2009 Jun 22.
9. Gonzalez AA, Lara LS, Luffman C, Seth DM, Prieto MC. Soluble form of the (pro)renin receptor is augmented in the collecting duct and urine of chronic angiotensin II-dependent hypertensive rats. *Hypertension*. 2011 Apr;57(4):859-864. Epub 2011 Feb 14.
10. Casarini DE, Boim MA, Stella RC, et al. Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. *Am J Physiol*. 1997 Mar;272(3 Pt 2):F405-F409.
11. Casarini DE, Plavinik FL, Zanella MT, et al. Angiotensin converting enzymes from human urine of mild hypertensive untreated patients resemble the N-terminal fragment of human angiotensin I-converting enzyme. *Int J Biochem Cell Biol*. 2001 Jan;33(1):75-85.
12. Von Thun AM, Vari RC, el-Dahr SS, Navar LG. Augmentation of intrarenal angiotensin II levels by chronic angiotensin II infusion. *Am J Physiol*. 1994 Jan;266(1 Pt 2):F120-F128.
13. Siragy HM, Carey RM. Protective role of the angiotensin AT2 receptor in a renal wrap hypertension model. *Hypertension*. 1999 May;33(5):1237-1242.
14. Nishiyama A, Seth DM, Navar LG. Renal interstitial fluid angiotensin I and angiotensin II concentrations during local angiotensin-converting enzyme inhibition. *J Am Soc Nephrol*. 2002 Sep;13(9):2207-2212.
15. Nishiyama A, Seth DM, Navar LG. Angiotensin II type 1 receptor-mediated augmentation of renal interstitial fluid angiotensin II in angiotensin II-induced hypertension. *J Hypertens*. 2003 Oct; 21(10):1897-1903.
16. Guan S, Fox J, Mitchell KD, Navar LG. Angiotensin and angiotensin converting enzyme tissue levels in two-kidney, one clip hypertensive rats. *Hypertension*. 1992 Dec;20(6):763-767.
17. Krebs C, Hamming I, Sadaghiani S, et al. Antihypertensive therapy upregulates renin and (pro)renin receptor in the clipped kidney of Goldblatt hypertensive rats. *Kidney Int*. 2007 Sep;72(6):725-730. Epub 2007 Jun 27.
18. Muller DN, Klanke B, Feldt S, et al. (Pro)renin receptor peptide inhibitor ''handle-region'' peptide does not affect hypertensive nephrosclerosis in Goldblatt rats. *Hypertension*. 2008 Mar;51(3): 676-681. Epub 2008 Jan 22.
19. Prieto MC, Williams DE, Liu L, Kavanagh KL, Mullins JJ, Mitchell KD. Enhancement of renin and prorenin receptor in collecting duct of Cyp1a1-Ren2 rats may contribute to development and progression of malignant hypertension. *Am J Physiol Renal Physiol*. 2011 Feb;300(2):F581-F588. Epub 2010 Nov 10.
20. Cousin C, Bracquart D, Contrepas A, et al. Soluble form of the (pro)renin receptor generated by intracellular cleavage by furin is secreted in plasma. *Hypertension*. 2009 Jun;53(6):1077-1082. Epub 2009 Apr 20.

This article meets the Accreditation Council for Graduate Medical Education and the American Board of Medical Specialties Maintenance of Certification competencies for Patient Care and Medical Knowledge.